

The Structure of a Human Type III Fc γ Receptor in Complex with Fc*

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Fc γ receptors mediate antibody-dependent inflammatory responses and cytotoxicity as well as certain autoimmune dysfunctions. Here we report the crystal structure of a human Fc receptor (Fc γ RIIIB) in complex with an Fc fragment of human IgG1 determined from orthorhombic and hexagonal crystal forms at 3.0- and 3.5-Å resolution, respectively. The refined structures from the two crystal forms are nearly identical with no significant discrepancies between the coordinates. Regions of the C-terminal domain of Fc γ RIII, including the BC, C'E, FG loops, and the C' β -strand, bind asymmetrically to the lower hinge region, residues Leu²³⁴-Pro²³⁸, of both Fc chains creating a 1:1 receptor-ligand stoichiometry. Minor conformational changes are observed in both the receptor and Fc upon complex formation. Hydrophobic residues, hydrogen bonds, and salt bridges are distributed throughout the receptor-Fc interface. Sequence comparisons of the receptor-ligand interface residues suggest a conserved binding mode common to all members of immunoglobulin-like Fc receptors. Structural comparison between Fc γ RIII-Fc and Fc ϵ RI-Fc complexes highlights the differences in ligand recognition between the high and low affinity receptors. Although not in direct contact with the receptor, the carbohydrate attached to the conserved glycosylation residue Asn²⁹⁷ on Fc may stabilize the conformation of the receptor-binding epitope on Fc. An antibody-Fc γ RIII model suggests two possible ligand-induced receptor aggregations.

Fc receptors, which are expressed on the majority of hematopoietic cells, play important roles in antibody-mediated immune responses. The binding of antigen-bound immunoglobulins (Ig) to Fc receptors activates their effector functions and leads to phagocytosis, endocytosis of IgG-opsonized particles, as well as antibody-dependent cellular cytotoxicity. The three major types of Fc receptors are Fc γ , Fc ϵ , and neonatal Fc receptors. Except for the neonatal Fc receptor and Fc ϵ RII (CD23), which are related structurally to class I major histocompatibility antigens and C-type lectins, respectively, all

other known Fc receptors are members of the immunoglobulin superfamily (1, 2). Among them, Fc γ RI and Fc ϵ RI¹ are high affinity Fc receptors for IgG and IgE, respectively, with dissociation constants ranging from 10⁻⁸ to 10⁻¹⁰ M. All other receptors for IgG, such as Fc γ RII and Fc γ RIII, are low affinity receptors with dissociation constants ranging from 10⁻⁵ to 10⁻⁷ M (3–5). In addition to variations in affinity, each receptor displays distinct IgG subtype specificities. Unlike the high affinity receptors that can bind monomeric antibodies, the low affinity receptors preferentially bind to and are activated by immune complexes.

Human Fc γ RIII exists as two isoforms, Fc γ RIIIA and Fc γ RIIIB, that share 96% sequence identity in their extracellular immunoglobulin-binding regions. Fc γ RIIIA is expressed on macrophages, mast cells, and natural killer cells as a transmembrane receptor. In contrast, Fc γ RIIIB, present exclusively on neutrophils, is anchored by a glycosyl-phosphatidylinositol linker to the plasma membrane. Although Fc γ RIIIA associates with the immunoreceptor tyrosine-based activation motif containing Fc ϵ RI γ -chain or the T cell receptor ζ -chain for its signaling, Fc γ RIIIB lacks a signaling component. Nevertheless, it plays an active role in triggering Ca²⁺ mobilization and in neutrophil degranulation (6, 7). In addition, Fc γ RIIIB, in conjunction with Fc γ RIIA, activates phagocytosis, degranulation, and the oxidative burst that leads to the clearance of opsonized pathogens by neutrophils. A soluble form of Fc γ RIIIB was reported to activate the CR3 complement receptor-dependent inflammatory process (8).

The Fc binding region on Fc γ RII and Fc γ RIII has been identified through the work of chimeric receptors with Fc ϵ RI as primarily the membrane proximal domain, including both the BC and FG loops. Further site-directed mutations have revealed several residues of the receptor critical to Fc binding (9–11). Similar regions on the α -chain of Fc ϵ RI were also identified to be critical for IgE binding affinity (12). The receptor binding site on Fc has been located through the construction of chimeric IgG molecules and mutational analysis at the lower hinge region, residues located in the hinge region between the C_H1 and C_H2 domains and immediately adjacent to the N terminus of the C_H2 domain of IgG (13–15). In particular, residues 234–238 (Leu-Leu-Gly-Gly-Pro) of the lower hinge of IgG1 have been implicated in the receptor binding. The corresponding region of IgE has also been implicated in the Fc ϵ RI binding (16). Apart from the lower hinge region, a few residues on the C_H2 domain of an IgG2b were also suggested to interact

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¹ The abbreviations used are: Fc γ RI and Fc ϵ RI, Fc receptors for IgG and IgE of the immunoglobulin superfamily; r.m.s., root mean square; FcRn, neonatal Fc receptor; RF, rheumatoid factors.

TABLE I
Data collection and refinement statistics

Data collection	Orthorhombic form		Hexagonal form
	Crystal 1	Crystal 2	
Space group	$P2_12_12_1$	$P2_12_12_1$	$P6_522$
Unit cell parameters (Å)	$a = 73.8$ $b = 102.4$ $c = 123.1$	$a = 76.4$ $b = 102.8$ $c = 123.3$	$a = b = 114.9$ $c = 301.4$
Resolution limit (Å)	3.0	3.3	3.5
Unique reflections	19063 (1859) ^a	13216 (1286)	15541 (1511)
Redundancy	4.8 (4.6)	3.0 (2.8)	4.7 (4.8)
Completeness (%)	99.9 (99.8)	86.4 (86.1)	98.7 (99.4)
$R_{\text{sym}} (\%)^b$	6.1 (46.0)	12.5 (46.5)	8.7 (42.3)
$I/\sigma(I)$	21.4 (3.6)	9.7 (2.5)	20.2 (4.0)
Refinement			
Resolution (Å)	10–3.0	10–3.3	10–3.5
No. reflections	17780	11948	14032
No. nonhydrogen atoms	4722	4733	4712
No. atoms in carbohydrate moieties	202	195	149
$R_{\text{cryst}} (\%)$	23.0 (39.3)	23.5 (31.3)	24.9 (36.5)
$R_{\text{free}} (\%)^c$	28.9 (50.8)	27.4 (36.5)	32.6 (47.7)
Mean B -factor (Å ²)	81	41	67
Wilson B -factor (Å ²)	82	54	82
r.m.s.d. bond lengths (Å)	0.011	0.008	0.009
r.m.s.d. bond angles (°)	1.82	1.51	1.69

^a Values for highest resolution shells, 3.00–3.11 and 3.30–3.44 Å for orthorhombic and 3.50–3.60 Å for hexagonal forms, are given in parentheses.

^b $R_{\text{sym}} = 100 \times \sum |I_h - \langle I_h \rangle| / \sum I_h$, where $\langle I_h \rangle$ is the mean intensity of multiple measurements of symmetry equivalent reflections.

^c R_{free} is calculated using test set of 5% of the reflections for the orthorhombic and 3% for hexagonal data sets.

with the receptor (17). However, with the exception of the neonatal Fc receptor, the molecular recognition between the Fc receptors and Fc remains to be elucidated (18).

The recent crystal structures of FcεRIα, FcγRIIA, and FcγRIIB have each revealed a conserved Ig-like structure, with particularly the small hinge angle between the two Ig-like domains, which is unique to the Fc receptors (19–21). We report here the crystal structure of a human FcγRIII in complex with the Fc portion of a human IgG1 determined from two crystal forms.

EXPERIMENTAL PROCEDURES

Protein Expression, Purification, and Crystallization—The extracellular part of the human FcγRIIIb receptor, residues 1–172, was expressed as *Escherichia coli* inclusion bodies and then reconstituted *in vitro* as described previously (22). Fc fragments of human IgG1 antibody were prepared by the papain digestion as previously reported (23, 24).

The complex of Fc and FcγRIII was prepared by mixing both components in a 1:1 molar ratio and concentrating to 8–15 mg/ml for crystallization. Single crystals of orthorhombic and hexagonal forms were obtained by vapor diffusion in hanging drops at room temperature under slightly different crystallization conditions. Rod-shaped crystals of the orthorhombic form were grown from 10% polyethylene glycol 4000 and 50 mM Hepes at pH 6.5. They appeared after 2–3 days and grew to an average size of $0.05 \times 0.05 \times 0.2$ mm in ~2 weeks. Crystals of the second form, hexagonal bipyramids, were crystallized from 5% polyethylene glycol 6000 and 50 mM Hepes at pH 6.0. Crystals were first observed after 4–5 days, and reached a maximum size $0.15 \times 0.15 \times 0.4$ mm after 1 month.

Structure Determination—After briefly soaking in precipitant solutions containing 25% glycerol, crystals were flash frozen at 100 K. X-ray diffraction data from single crystals of both crystal forms were collected using an ADSC Quantum IV charge-coupled device detector at the X9B beam line of the National Synchrotron Light Source at the Brookhaven National Laboratory and processed with HKL2000 (25). The hexagonal crystals belong to space group $P6_522$ with cell dimensions $a = b = 114.9$ and $c = 301.4$ Å and diffract to 3.5 Å. Due to the large unit cell dimension along c in the hexagonal crystals, data were collected at a 300-mm crystal to detector distance with a small oscillation angle of 0.2°. The orthorhombic crystals of space group $P2_12_12_1$ with cell dimensions of $a = 76.4$, $b = 102.8$, and $c = 123.3$ Å diffracted to 3.0 Å. Both crystal forms contain one molecule of FcγRIII and one molecule of Fc in the asymmetric unit.

The structure of the FcγRIII-Fc complex in both crystal forms was determined by molecular replacement. Polyalanine models of Fc (PDB

accession number 1FC1) and FcγRIII were used in rotation and translation searches using 15–4 Å data for both crystal forms. An $I/\sigma(I)$ cutoff of 2.0 was used for hexagonal data during search procedures. The position of the Fc molecule was determined in both crystal forms using AmoRe (26). Rotation and translation searches using data from the orthorhombic crystal resulted in an unambiguous solution with a correlation coefficient (CC) of 39% and an R -factor (R_p) of 51% (CC = 51% and R_p = 49% after rigid-body refinement in AmoRe). Molecular replacement searches using the hexagonal crystal data yielded a solution for the Fc from the third highest rotation solution that became the highest ranking translation solution with CC = 38% and R_p = 52% (CC = 46% and R_p = 50% after rigid-body refinement in AmoRe). The position of FcγRIII was determined in both crystal forms using the program EPMR (27) with a polyaniline model of FcγRIII and the position of the Fc molecule fixed. Clear solutions were obtained for both crystal forms with CC = 56% and R_p = 48% for orthorhombic crystal and CC = 55% and R_p = 48% for hexagonal crystal, respectively. After rigid-body refinement of individual domains of the FcγRIII-Fc complex modeled as polyaniline using CNS (28) most side chains had clear electron density into which side chains were built in. Disordered side chains lacking electron density were built with occupancies set to zero. The positional and grouped B -factor refinement was carried out using maximum likelihood as a target function with CNS version 0.9. Model adjustments and rebuilding were done using the program O (29). Carbohydrate molecules were added manually using $2F_o - F_c$ electron density maps contoured at 1.0σ and refined. The final model includes residues 5–172 of FcγRIII, residues 235–444 for one chain of Fc, and residues 233–443 for the other chain of Fc.

RESULTS AND DISCUSSION

Overall Structure of the Complex—Crystals of a human FcγRIII receptor in complex with a human Fc fragment of IgG1 were grown in two forms under different conditions. The orthorhombic crystals belong to the space group of $P2_12_12_1$ and diffract to 3.0-Å resolution, whereas the hexagonal crystals have $P6_522$ space group symmetry and diffract to 3.5-Å resolution. The structure of the complex was determined by molecular replacement in both forms and refined to their resolution limit. The final R -factors are $R_{\text{cryst}} = 23.0\%$ and $R_{\text{free}} = 28.9\%$ for the orthorhombic form and $R_{\text{cryst}} = 24.9\%$ and $R_{\text{free}} = 32.6\%$ for the hexagonal form, respectively (Table I). The electron density is continuous throughout the complex in the final $2F_o - F_c$ map except for three surface loops of FcγRIII (residues 31–34, 99–105, and 142–149) located opposite from the Fc interface region. Despite different crystal packing and solvent

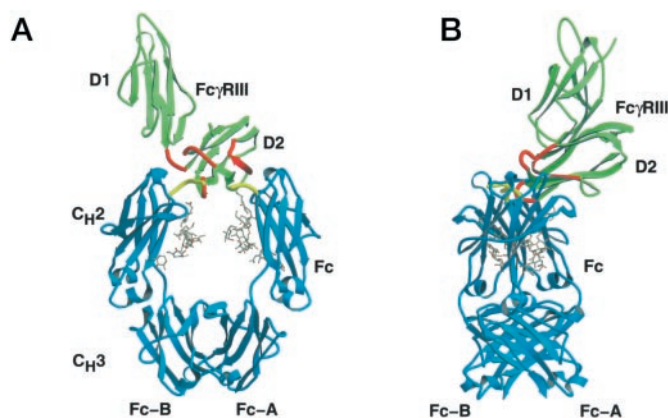


FIG. 1. **Ribbon drawing showing front view (A) and side view (B) of the Fc γ RIII-Fc.** The side view of the complex is rotated $\approx 90^\circ$ from the front view. Fc γ RIII is shown in green, Fc is cyan. Positions of D1 and D2 domains of Fc γ RIII as well as C_H2 and C_H3 domains of Fc are marked. Carbohydrate moieties are shown in gray.

contents (57% in the orthorhombic and 64% in the hexagonal form), both crystals contain one Fc γ RIII and one Fc molecule in each asymmetric unit, suggesting a 1:1 stoichiometry for the binding between the receptor and Fc (Fig. 1). This is consistent with earlier binding studies using non-equilibrium and equilibrium gel filtration experiments (22). The conformation of the Fc γ RIII-Fc complex is essentially identical in both crystal forms, including the conformation of the visible carbohydrate moieties on the Fc fragment (Fig. 2A).

The Structure of Fc γ RIII—The structure of Fc γ RIII in both the orthorhombic and the hexagonal crystal forms can be readily superimposed with the structure of ligand free receptor resulting in r.m.s. differences between the individual domains of 0.6–0.8 Å among all C α atoms (Fig. 2B) (22). The hinge angle between the N-terminal (D1) and the C-terminal (D2) domains is 60° , which is slightly larger than the 50° value observed in the ligand free receptor. However, no significant change in the receptor conformation is observed upon complex formation (Fig. 2B).

The Structure of Fc—The Fc fragment of an IgG1 antibody comprises two identical chains (A and B), and each consists of two C1-type immunoglobulin domains, C_H2 and C_H3. The overall shape of the Fc fragment resembles that of a horseshoe with the two C_H3 domains packing tightly against each other at the bottom of the horseshoe and the C_H2 domains held apart by carbohydrate moieties attached to the glycosylation site Asn²⁹⁷ from both chains forming the opening of the horseshoe. Well defined electron density throughout the Fc allowed for unambiguous tracing of residues Leu²³⁴ to Ser⁴⁴⁴ in chain A and Pro²³² to Leu⁴⁴³ in chain B of Fc, including the lower hinge regions, Leu²³⁴–Pro²³⁸. The structure of the Fc fragment in complex with Fc γ RIII does not differ significantly from that observed in the structures of an unbound Fc fragment and a murine intact IgG2a antibody (30, 31) (Fig. 2C). However, the 2-fold symmetry relating the two chains of Fc in other unligated Fc structures, is no longer retained in the structure of the complex. The horseshoe-shaped Fc is slightly more open at the N-terminal end of the C_H2 domains in the Fc γ RIII-Fc structure compared with other known structures of Fc. The hinge angle between C_H2 and C_H3 domains of chain A (Fc-A) is 95° and 100° in the orthorhombic and the hexagonal crystals, respectively, $\sim 10^\circ$ larger than the corresponding angle of chain B (Fc-B) and the 84° – 89° angle observed in all structures of ligand-free Fc (Fig. 2C).

The Interface between Fc γ RIII and Fc—The receptor binds to Fc at the center of the horseshoe opening making contacts to

the lower hinge regions of both A and B chains of Fc (designated here as Hinge-A and Hinge-B, respectively, for the lower hinges of Fc-A and Fc-B) (Fig. 1). Such binding breaks down the dyad symmetry of the Fc, creating an asymmetric interface whereby the identical residues from Hinge-A and Hinge-B interact with different, unrelated surfaces of the receptor. Furthermore, it excludes the possibility of having a second receptor interacting with the same Fc molecule, resulting in a 1:1 stoichiometry for the receptor-Fc recognition. The structural implications of the activation of Fc receptors is profound. Particularly, the 1:1 receptor-Fc binding stoichiometry highlights the importance of antigen in the receptor aggregation. In contrast to the high affinity Fc γ RI and Fc ϵ RI receptors, the binding of immunoglobulins to Fc γ RIII in the absence of antigen does not lead to receptor aggregation. It can be argued that a 1:1 receptor-ligand stoichiometry ensures the need for antigens in forming the receptor aggregation by eliminating the possibility of Fc-mediated receptor aggregation as suggested in a 2:1 stoichiometry. Precluding receptor aggregation mediated by Fc alone also eliminates the potential deleterious effect of antibodies whose concentration *in vivo* are often much higher than that of antigen.

The receptor-Fc complex buries ~ 1453 Å² of solvent-accessible area (Fig. 3A). The interface between Fc γ RIII and Fc molecules shows poor shape complementarity with a mean shape correlation statistic of 0.53 (32), less than those between T-cell antigen receptor and Class I major histocompatibility complex molecules, between adhesion receptor CD2 and CD58, and between antibody and antigen complexes. On the receptor side, all the contacts to Fc are made exclusively through its D2 domain. The receptor D1 domain is positioned above the Fc-B and makes no contacts with Fc (Fig. 1A). The interface of the complex consists of the hinge loop between the D1 and D2 domain of the receptor, the BC, C'E, and FG loops, and the C' β -strand. The BC loop is positioned across the horseshoe opening making contact with residues of both Hinge-A and Hinge-B. The C'-strand is situated atop the Fc-A leading to the C'E loop in contact with residues of Hinge-A. The FG loop of Fc γ RIII protrudes into the opening between the two chains of Fc (Fig. 1A). All three receptor loops (BC, C'E, and FG) were implicated in Fc binding through earlier studies of chimeric Fc γ RII/Fc ϵ RI receptors and through site-directed mutagenesis (9, 10, 12). On the Fc side of the complex, interactions with the receptor are dominated by residues Leu²³⁴–Pro²³⁸ of the lower hinge (Table II), consistent with results from earlier mutational studies (2). In particular, Hinge-A and -B together contribute $\sim 60\%$ of the overall receptor-Fc interface area (Fig. 3A). Interestingly, both Hinge-A and -B are found disordered in all known Fc structures to date, including the structure of an intact mouse IgG2a (30, 33–35). In contrast, residues of both Hinge-A and -B are clearly visible in the electron density maps from both crystal forms, suggesting that the binding of Fc γ RIII stabilizes the lower hinge conformation of Fc.

A combination of salt bridges, hydrogen bonds, and hydrophobic interactions contributes to the receptor-Fc recognition. Specifically, the interface between Fc γ RIII and Fc-A is dominated by hydrogen bonding interactions, whereas the hydrophobic interactions occur primarily at the interface between Fc γ RIII and Fc-B. There are a total of nine hydrogen bonds between the receptor and Fc, forming an extensive network involving both the main-chain and side-chain hydrogen bonding interactions (Fig. 3, B and C, and Table II). Seven hydrogen bonds are distributed across the receptor and Fc-A interface and two are at the receptor and Fc-B interface. Alanine mutations, such as the H134A mutant of Fc γ RII that resulted in the loss of two interface hydrogen bonds, have been shown to re-

FIG. 2. *A*, superposition of an α -carbon trace of the Fc γ RIII-Fc complex determined from both the orthorhombic (green and cyan for Fc γ RIII and Fc, respectively) and hexagonal (orange and red) crystal forms. *B*, superposition of the structure of Fc γ RIII in the receptor-Fc complex (green) with that of ligand free receptor (orange). *C*, superposition of the structure of Fc in Fc γ RIII-Fc complex (cyan) with that of unbound Fc (red).

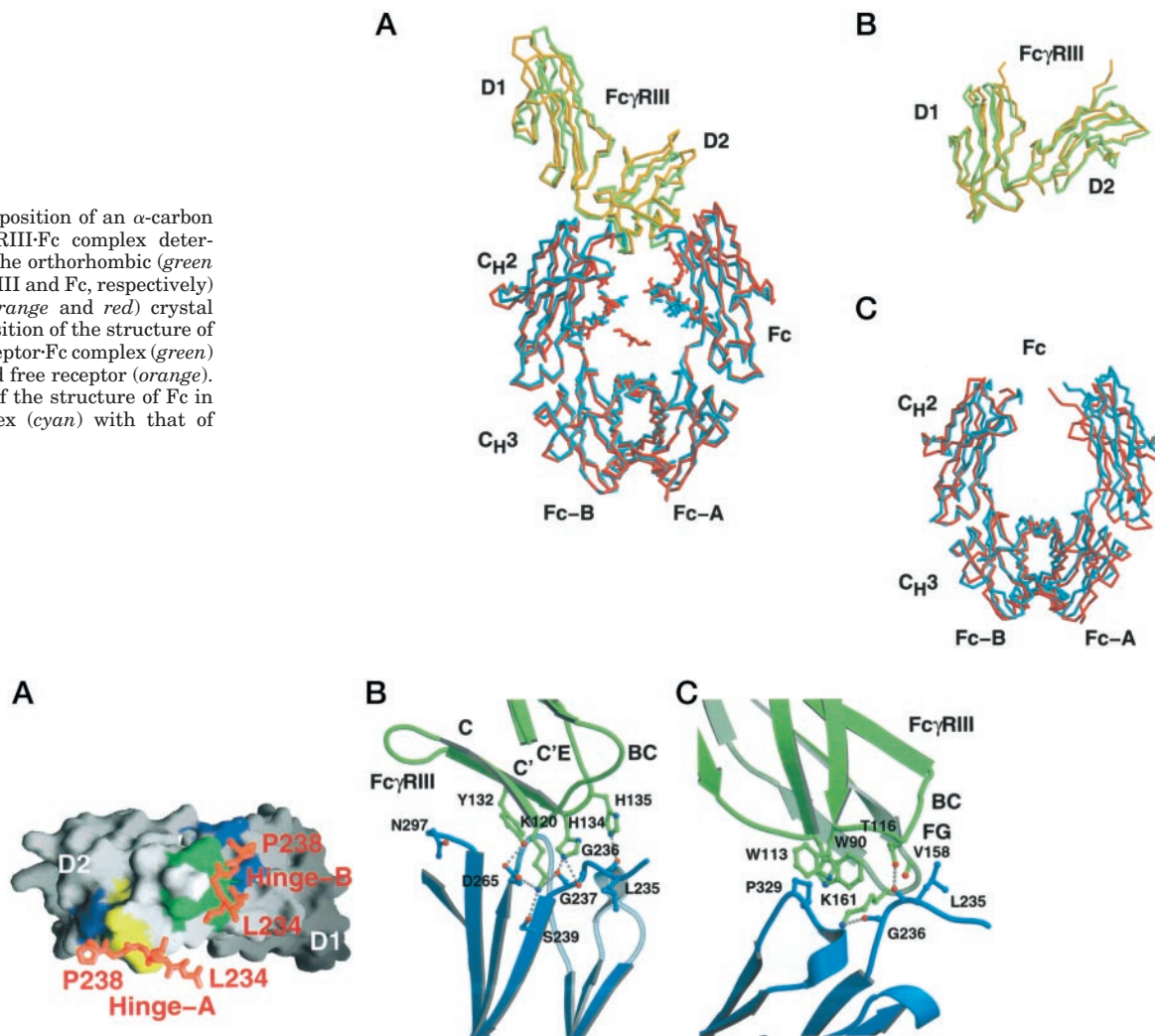


FIG. 3. **Fc γ RIII-Fc interface.** *A*, surface representation of Fc γ RIII. The interface region is color-coded. Regions involved in the interactions with Hinge-A and Hinge-B are yellow and green, respectively. All other contact areas are colored in blue. Lower hinge regions of Fc are in ball-and-stick representation (red). *B*, interactions between Fc γ RIII (green) and chain A of Fc (cyan). The glycosylation residue, Asn²⁹⁷, is also shown. *C*, interactions between Fc γ RIII (green) and chain B of Fc (cyan). The side chain of Val¹⁵⁸ is omitted for clarity. There is a hydrogen bond between carbonyl group of Val¹⁵⁸ and backbone nitrogen of Gly²³⁶ (Table II) that is not shown in the picture. Residues are colored by molecule. Important hydrogen bonds are represented by dotted lines. The BC, C'E, and FG loops as well as the C and C' β -strands of Fc γ RIII, which play an important role in the interactions, are labeled. Some secondary structure elements of Fc lying behind and not contributing to the binding shown as semi-transparent. Carbohydrate moieties have been omitted for clarity.

duce the receptor-Fc binding drastically, illustrating the importance of the interface hydrogen bonding network to the stability of the complex (10). A hydrophobic core is formed between Trp⁹⁰, Trp¹¹³ of the receptor, and Pro³²⁹ of the C_H2 domain of Fc-B (Fig. 3C). This hydrophobic core extends further to include Val¹⁵⁸, the aliphatic side chain of Lys¹⁶¹ of the receptor and Leu²³⁵ of Hinge-B. Mutations of both Trp¹¹³ and Lys¹⁶¹ in Fc γ RIII lead to the loss in receptor function (11, 36). The side chain of Leu²³⁵ on the Fc-B packs tightly against Gly¹⁵⁹ of the receptor leaving little space to accommodate any residues larger than Gly at this position. A G159A mutation on chimeric Fc γ RII resulted in the complete disruption of Fc binding, presumably due to the steric hindrance between Leu²³⁵ and the β -carbon of the alanine mutant at position 159 (9). Of particular interest is Trp¹¹³ of the receptor, which when mutated to Phe resulted in the loss of Fc binding. This residue is not only part of the interface hydrophobic core but also functions as a wedge inserted into the D1 domain to stabilize the acute interdomain hinge angle between D1 and D2 domains of Fc γ RIII. A W113F mutation would result in the loss of this wedge and lead to a disruption in binding by altering the orientation between the D1 and D2 domains.

Comparison of the Structures of Receptor-Fc Complexes— Including the two crystal forms described in this work, there are a total of four Fc receptor and Fc complex structures available to date. A comparison among these structures reveals the conformation flexibility of this receptor-ligand complex and helps to explain the molecular interactions that differentiate the high from the low affinity receptors.

The two crystal forms of Fc γ RIII-Fc complexes determined in the present study are essentially identical and can be readily superimposed with a root mean square (r.m.s.) deviations of 1.1 Å among all C α atoms. The superposition of the hexagonal form onto the published Fc γ RIII-Fc complex resulted in an r.m.s. deviation of 0.5 Å for all C α atoms (37) (Fig. 4A). An analysis of the interdomain hinge angles shows that the C_H2-C_H3 hinge angle is 10° larger in the structure of the Fc γ RIII-Fc complex than it is in the structure of an intact IgG2a antibody (35) or the structures of ligand-free Fc (30) (Table III). This results in a slightly more open conformation of the Fc when ligated to the receptor. Apart from the small change of the hinge angle, neither the Fc nor the receptor displays significant conformational change upon complex formation. The agreement between the orthorhombic and hexagonal crystal forms of the complex

TABLE II
Interface contacts between FcγRIII and Fc

FcγRIII	Fc	Chain	Distance (Å)
Hydrogen bonds and salt bridges			
Thr ¹¹⁶ O _{γ1}	Leu ²³⁵ O	B	3.3
Lys ¹²⁰ N _ε	Ser ²³⁹ O _γ	A	3.4
Lys ¹²⁰ N _ε	Asp ²⁶⁵ O _{δ1}	A	3.2
Lys ¹²⁰ N _ε	Gly ²³⁷ O	A	3.4
His ¹³⁴ N _{δ2}	Gly ²³⁶ O	A	3.5
His ¹³⁴ N _{ε2}	Gly ²³⁷ O	A	2.4
His ¹³⁵ N _{ε2}	Leu ²³⁵ O	A	3.2
Val ¹⁵⁸ O	Gly ²³⁶ N	B	3.5
Lys ¹⁶¹ N _ε	Gly ²³⁶ O	B	3.0
Hydrophobic contacts ^a			
Ile ⁸⁸	Ala ³³⁰	B	
Trp ⁹⁰	Pro ³²⁹ , Gly ²³⁶	B	
Trp ¹¹³	Pro ³²⁹	B	
His ¹³⁴	Gly ²³⁶ , Gly ²³⁷ , Asp ²⁶⁵	A	
Val ¹⁵⁸	Leu ²³⁵	B	
Gly ¹⁵⁹	Leu ²³⁵	B	
Lys ¹⁶¹	Gly ²³⁶	B	

^a Carbon-carbon contacts ≤ 4.0 Å.

indicates a well defined receptor-ligand recognition free from conformational flexibility.

The comparison between the structure of the FcγRIII-Fc complex and that of the FcεRI-Fc complex has provided further insight into the molecular basis of the receptor affinity (38). Overall, a similar mode of receptor-ligand recognition was observed in both the FcγRIII-Fc and the FcεRI-Fc complexes with an r.m.s. deviation of 1.5 Å between all the Cα atoms. In fact, most of the structural difference resulted from the small variation between the C_H2-C_H3 and Cε3-Cε4 interdomain hinge angles (Fig. 4, B and C). This angle is ~10° smaller in the FcεRI-Fc complex structure, resulting in a slightly closed conformation of Fc compared with that of the FcγRIII-Fc complex.

Detailed structural analysis shows that the interface area buried in the high affinity FcεRI-Fc complex (1850 Å²) is 400 Å² more than that in the low affinity FcγRIII-Fc complex (1453 Å²). This is primarily due to a more extensive interaction observed between the receptor and the non-lower hinge residues of Fc in the high affinity complex than in the low affinity receptor complex. Of the total interface area of the Fc, the lower hinge and non-lower hinge regions contribute 870 and 580 Å², respectively, in the FcγRIII-Fc structure. The corresponding regions contribute 740 and 1110 Å², respectively, in the FcεRI-Fc structure. This results in approximately twice as much interface area contributed by non-lower hinge residues in the high affinity receptor-ligand complex than in the low affinity receptor-ligand complex. Structurally, the lower hinge of IgE-Fc adopts a very different conformation than that of IgG-Fc in their respective receptor complexes (Fig. 4D). This conformation difference may enable the high affinity FcεRI to interact more extensively with its ligand.

Although the overall pattern of the receptor-Fc interactions, namely a preference for hydrogen bonding in the Fc A-chain part of the interface and hydrophobic contacts in the Fc B-chain part of the interface are preserved in both the FcγRIII-Fc and FcεRI-Fc complexes, significant differences were also observed. First, there are more extensive hydrophobic interactions between FcεRI and IgE-Fc than those between FcγRIII and IgG-Fc. Although the tryptophan-proline sandwich formed by Trp⁹⁰, Trp¹¹³ of FcγRIII and Pro³²⁹ of Fc-B (the corresponding Trp⁸⁷, Trp¹¹⁰, and Pro⁴²⁶ residues in FcεRI-Fc) is preserved in both structures, additional bulky residues, such as Trp¹³⁰, found in both FcεRI and IgE-Fc may also contribute to stronger hydrophobic interactions in the FcεRI-Fc complex compared

with that of the FcγRIII-Fc complex. Second, a more extensive network of hydrogen bonds and salt bridges exists at the FcεRI-Fc interface compared with that of FcγRIII-Fc. Furthermore, the hydrogen bonds in the FcεRI-Fc interface are formed mostly between the side-chain atoms whereas those in the FcγRIII-Fc interface are formed primarily between the main-chain atoms or between the main-chain and side-chain atoms. There are two salt bridges Lys¹¹⁷-Asp³⁶² and Glu¹³²-Arg³³⁴ observed between FcεRI and Fc but only one, Lys¹²⁰-Asp²⁶⁵, is conserved in the low affinity complex between FcγRIII and Fc.

Our results suggest that multiple interactions contribute to the observed receptor-ligand affinity difference and that the higher affinity recognition includes more extensive hydrophobic interface area as well as more prominent electrostatic interactions.

Conserved Receptor-Fc Binding Interface—Of the 13 receptor interface residues, four (Trp⁹⁰, Trp¹¹³, Lys¹³¹, and Gly¹⁵⁹) are invariant among all human Fcγ receptors (Fig. 5A). Three of them are also conserved in the α-chain of FcεRI. Gly¹⁵⁹ in Fcγ receptors is replaced with Trp in FcεRI. Because Gly¹⁵⁹ is in close contact with Leu²³⁵ from the lower hinge of Fc-B, replacement of this residue with Trp may result in the observed difference of the lower hinge conformation in IgE. Three other interface residues, Lys¹²⁰, Tyr¹³², and Val¹⁵⁸, are nearly invariant among all human Fc receptors. The limited variation observed can be easily modeled into the existing interface without creating steric hindrance. It is interesting that the interface salt bridge between Lys¹²⁰ and Asp²⁶⁵ of the Fc appears to be absent in FcγRI but conserved in all other Fcγ receptors and in FcεRI. The other six interface residues, Ile⁸⁸, Asp¹²⁹, His¹³⁴, His¹³⁵, Arg¹⁵⁵, and Lys¹⁶¹ are less well conserved among the receptors. Of these, variation at His¹³⁴ and His¹³⁵ may result in conformational changes in the lower hinge region of bound Fc. Overall, key features of the receptor-Fc interface appear to be well preserved among all the Fc receptors with possible hinge conformational adjustment for each receptor-Fc pair. Of particular interest is the comparison between the interface residues of FcγRI and those of FcγRIII. The binding affinity of FcγRIII is at least 100-fold weaker than that of FcγRI. Among the receptor-Fc interface residues, only four are different between FcγRIII and FcγRI. These are Lys¹²⁰, Tyr¹³², Arg¹⁵⁵, and Lys¹⁶¹ in FcγRIII and Asn¹²⁰, Phe¹³², Ser¹⁵⁵, and His¹⁶¹ in FcγRI. It is, however, not clear if any of these residues contribute to the observed variation in binding affinity.

Fc Receptor IgG Subtype Specificities—Fcγ receptors display IgG subtype specificities. In particular, human FcγRIII binds tighter to IgG1 and IgG3 than it does to IgG2 and IgG4. Most of the Fc residues in contact with the receptor are conserved among the IgG sequences (Fig. 5B, residues boxed in blue and red), suggesting a conserved binding site for all human IgGs. These binding residues, with the exception of a Glu²⁶⁹ to Asp replacement, are also conserved in murine IgG2a consistent with it being a ligand for human Fcγ receptors. The sequence differences among the IgG subclasses exist primarily at the lower hinge region. First, hIgG2 has a Val and Ala at positions 234 and 235, respectively, instead of Leu and Leu as observed in IgG1 and IgG3, and a one-residue deletion at position 237 of the corresponding IgG1. Human IgG4 has a Phe at position 234 (Fig. 5B). In addition, IgG2 and IgG4 sequences contain a three-residue deletion relative to IgG1 at the N-terminal end of the lower hinge, possibly restricting the lower hinge conformation. The length of the lower hinge has been suggested as a factor in lower receptor binding affinity of IgG2 and IgG4 (2). Among the four IgG subtypes, the length of the hinge region is longest in IgG3 and shortest in IgG2 and IgG4 (three to four residues shorter than that of IgG1). The differences in both the

FIG. 4. **Superposition of the Fc γ RIII-Fc complex determined from the orthorhombic crystal form onto the structures of previously determined complexes Fc γ RIII-Fc (46) and Fc ϵ RI-Fc (45).** A, superposition of the Fc γ RIII-Fc complex determined from the orthorhombic crystal form (green and cyan for Fc γ RIII and Fc, respectively) and previously determined structure of Fc γ RIII-Fc complex (46) (orange and red for Fc γ RIII and Fc, respectively). B, superposition of the structure of Fc γ RIII-Fc (green and cyan for Fc γ RIII and Fc, respectively) and Fc ϵ RI-Fc (orange and red for Fc ϵ RI and Fc, respectively) complex (45). C, a definition of the hinge angles. D, an enlarged view of superposition of Fc γ RIII-Fc (green and cyan) and Fc ϵ RI-Fc (red and orange) complexes in the interface area. The lower hinge regions are labeled. The view is identical to that in B.

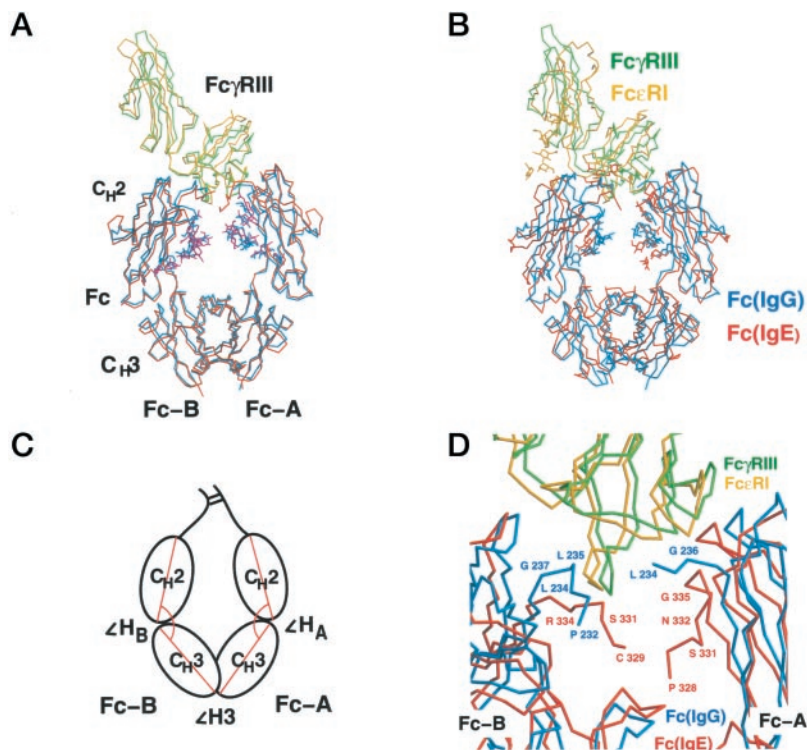


TABLE III

Hinge angle comparison of free and receptor complexed Fc

The hinge angles are defined in Fig. 4C.

	$\angle H_A$	$\angle H_B$	$\angle H_3$
IgG2a (35)	90	87	69
Fc (30)	87	83	68
Fc γ RIII-Fc (P2,2,2 ₁)	96	87	68
Fc γ RIII-Fc (P6 ₂ 22)	100	90	68
Fc γ RIII-Fc (37)	101	90	68
Fc ϵ RI-Fc (38) ^a	87	84	59

^a For the structure of Fc ϵ RI-Fc complex angles $\angle H_A$, $\angle H_B$ and $\angle H_3$ correspond to the angles between C ϵ 3 and C ϵ 4 domains for A and B chains and between C ϵ 4 domains, respectively.

amino acid composition and the length of lower hinge may contribute to the observed lower receptor binding affinity of IgG2 and IgG4.

The Contribution of Carbohydrate to the Fc γ Receptor-Fc Binding—Both Fc γ receptors and antibodies are glycosylated *in vivo*. In contrast to the Fc fragment that displays only one conserved carbohydrate attachment site located at Asn²⁹⁷, the receptor glycosylation sites vary both in number and in location among different Fc γ receptors. For example, the glycosylation sites on the C-terminal domain of Fc γ receptors are located at residues Asn¹⁶² and Asn¹⁶⁹ on Fc γ RIII, Asn¹³⁸ and Asn¹⁴⁵ on Fc γ RII and asparagines 138, 145, and 149 on Fc γ RI (Fig. 5A). The influence of glycosylation on the receptor-Fc binding kinetics and on the receptor function has been studied extensively using both the deglycosylated receptor and Fc (4, 39). These studies demonstrated that the carbohydrate attached to Asn²⁹⁷ of Fc have a significant impact on the receptor binding, whereas glycosylations on the receptors appeared less critical and perhaps have more of a modulating effect on the affinity. For example, the two neutrophil antigen A alleles of Fc γ RIIB, NA1 and NA2, differing primarily in their carbohydrate contents, display a 2-fold difference in their affinity for IgG3.

The structure of the receptor-Fc complex reveals potential roles for carbohydrate in receptor-Fc recognition. The first is the potential role of glycosylation at Asn²⁹⁷ in supporting the

structural framework of the Fc. The Fc fragment used in this work was generated from a human IgG1 and is therefore glycosylated. Multiple carbohydrate moieties were visible in the electron density extending from Asn²⁹⁷ of both chains of Fc toward each other into the inter-chain region, referred to as the carbohydrate core region. Asn²⁹⁷ is located next to the receptor binding interface. The carbohydrate moieties, however, are orientated away from the interface making no specific contacts with the receptor. The glycosylation is thus unlikely to influence the receptor-Fc interface directly. However, the unique arrangement between the oligosaccharide moieties and the polypeptide chains of Fc makes it possible for the carbohydrate to affect the conformational stability of the receptor binding epitopes (40). Specifically, the spacing and the orientation between the two C H_2 domains may be influenced by the presence of sugar attachments (Fig. 1A). Because the binding of the receptor to Fc requires a particular orientation of the epitopes on both chains of Fc, it makes the receptor-Fc interface sensitive to the relative position and orientation of the two C H_2 domains.

A Model for Fc γ RIII-IgG Recognition—On a cell surface, the Fc receptor recognizes intact immunoglobulins. The presence of the Fab portion of antibody is likely to impose restrictions to the receptor-Fc recognition. To date, the only structure of an intact antibody available is that of a mouse IgG2a (31). Because Fc γ RIII also recognizes mouse IgG2a, a model of this receptor-antibody complex was generated by superimposing the Fc part of the current structure onto the Fc of the IgG2a (Fig. 6A). This receptor-antibody recognition model reveals that the receptor fits tightly and is nearly engulfed by the bound antibody.

Although the current structure offers an insight to antibody-Fc γ receptor recognition, the mechanism of receptor activation, namely the antigen-driven receptor clustering, remains unknown. Two receptor clustering models can be proposed based on the current structural results, a simple avidity model and an ordered receptor aggregation model (Fig. 6, B and C). The simple avidity receptor activation model assumes that the binding of oligomeric antigens by antibodies increases the

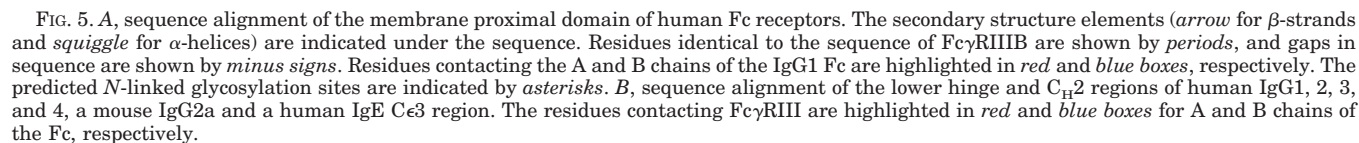
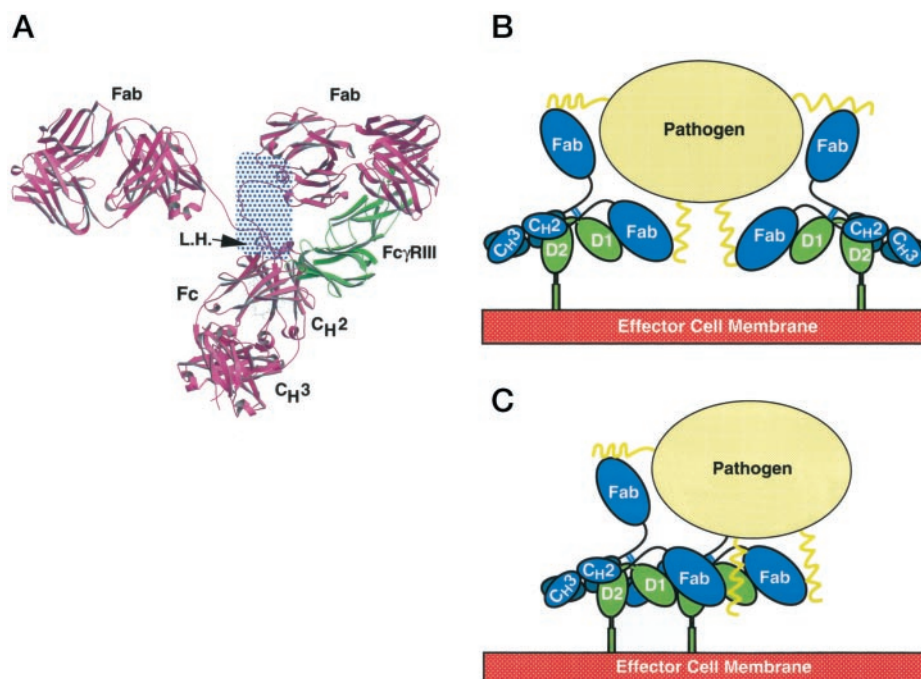


FIG. 6. **Antibody-Fc γ RIII binding and ligand induced receptor aggregation model.** A, an intact antibody-Fc γ RIII binding model. The structure of the antibody is shown in *magenta* and that of Fc γ RIII is in *green*. The position of the second possible orientation of Fc γ RIII, which is in direct steric conflict with the hinge region and Fab, is indicated by a *blue-shaded area*. The *arrow* points to the location of the lower hinge (*L.H.*). The Protein Data Bank entry for the antibody coordinates is 1IGT. B, a simple avidity model of antigen-antibody binding induced Fc γ RIII aggregation. C, an ordered receptor aggregation model.



avidity as well as the proximity of the receptors, which is sufficient to its activation. The ordered receptor aggregation model assumes that the binding of oligomeric antigens leads to the formation of an ordered receptor-ligand aggregation, which further stabilizes the activation complexes. Recent imaging studies on T cell and NK cell receptor activation processes suggest that the formation of the so-called immune synapse is an ordered event (41, 42). These results favor the structured aggregation model rather than the simple avidity model, although the molecular organization of Fc γ receptors during their activation remain to be determined. Recently, an ordered receptor-ligand aggregate was observed in the crystal lattice of a natural killer cell receptor in complex with its class I major histocompatibility complex ligand (43). Such a receptor-ligand aggregate is not observed in the two forms of the current Fc γ RIII-Fc crystals. However, a parallel receptor aggregate was observed in the crystal lattice of Fc γ RIII in the absence of Fc (22). A superposition of the current complex structure onto this lattice receptor aggregate suggests that the clustering model be compatible with the structure of a receptor-Fc complex (Fig. 6C).

Comparison of Fc γ Receptor with Other Ligands of Fc—The Fc region of the IgG molecule possesses multiple recognition sites for different components of immune system, including Fc γ receptors, neonatal Fc receptor (FcRn), rheumatoid factors (RF) and components of the complement system. In addition, it is also used as a ligand by staphylococcal proteins A and G. The structures of Fc complexed to FcRn, RF, protein A, and protein G are now known (30, 33, 34, 44). The binding of Fc by Fc γ receptors is characteristically different from all other known Fc ligands. First, the location of the Fc receptor binding site differs from those of neonatal Fc receptor, RF, and protein A. Although Fc γ receptors bind to the lower hinge region of Fc between the C_H1 and C_H2 domains, FcRn, RF, protein A, and protein G bind to the joint region between the C_H2 and C_H3 domains of Fc. Second, Fc γ receptors recognize Fc in an asymmetric fashion resulting in one receptor bound to both chains of Fc whereas all other ligands bind Fc in a symmetric fashion with each chain of Fc harboring an intact binding site (Fig. 7). The distinct binding site for Fc γ receptors suggests that it is possible to bind Fc γ receptors simultaneously with other ligands that recognize the C_H2-C_H3 joint region on the same Fc molecule. This raises the possibility of activation of multiple immune components by the same antigen-bound immune complex.

The recognition mode of binding to the lower hinge of Fc may evolve from the unique requirement of Fc γ receptor signaling, namely the need to have 1:1 recognition stoichiometry and to be capable of discriminating the IgG subtypes. Both C_H2 and C_H3 domains of Fc are very conserved among the subclasses of IgGs. Even the C_H2-C_H3 joint region, which is involved in binding of other Fc ligands, has near identical sequences among the IgG subclasses (Fig. 5B). The lower hinge region of IgGs, in comparison, is more variable allowing subtype-specific recognition of the receptor. The conformation of the hinge region, however, is quite flexible compared with the C_H2 and C_H3 domains of Fc (35). This hinge flexibility, which enables the Fab arms to adapt to the shape and form of antigens, may in fact hinder the binding of Fc receptors. Interestingly, there are two conserved cysteine residues forming two disulfide bonds at the N-terminal end of the lower hinge. The presence of these disulfides may stabilize the lower hinge conformation while allowing sufficient flexibility at the upper hinge region. Finally, the binding to the lower hinge region of both chains of Fc allows the receptor to monitor the integrity of the antibody.

Receptor-IgG Recognition and Autoimmune Diseases—In addition to their normal cellular functions in host immunity,

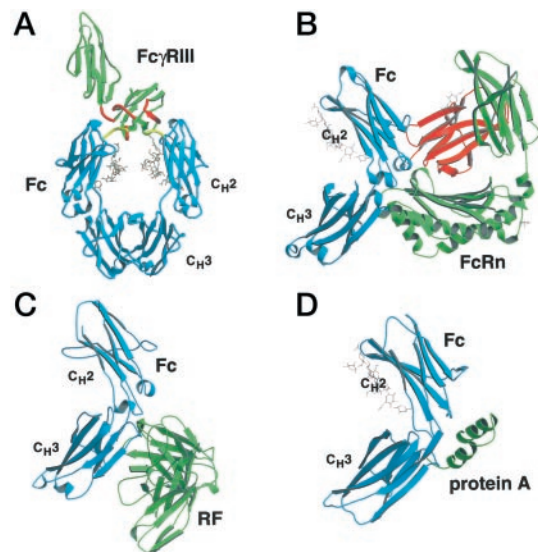


FIG. 7. Recognition of Fc by multiple ligands. Structural comparison among the complexes of (A) Fc γ RIII-Fc; (B) FcRn-Fc (PDB entry 1FRT); (C) rheumatoid factors RF-Fc (PDB entry 1ADQ); and (D) bacterial protein A-Fc (PDB entry 1FC2). Protein G binds similar to Fc as does protein A. Due to its symmetric interaction with ligand, only one chain of Fc is shown in the FcRn-Fc, RF-Fc and protein A-Fc complexes. The corresponding Fc regions are colored in cyan and shown in similar orientations. The ligands to Fc are colored in green and the β_2 -microglobulin of FcRn is shown in red. Only the variable domain of RF is shown. The carbohydrates are shown in ball-and-stick models.

Fc γ Rs, in particular Fc γ RI and Fc γ RIII, also mediate the inflammatory responses generated by cytotoxic autoantibodies and immune complex triggered inflammatory disorders (45, 46). They provide a critical link to autoimmune diseases, such as rheumatoid arthritis, hemolytic anemia, and thrombocytopenia. The structure of Fc γ RIII in complex with IgG1-Fc reveals the molecular interface of this receptor-Fc recognition and thus provides new possibilities for developing therapeutic reagents to block the activation of Fc receptors by autoantibodies. For example, the lower hinge sequence of Fc may be used to generate neutralizing antibodies that could block the binding of autoantibodies to Fc γ Rs. The peptides encompassing residues of the BC and FG loops of the C-terminal domain of Fc γ receptors could also be used to develop neutralizing antibodies against the receptors. Finally, reagents that affect the glycosylation pathway may be used to affect the carbohydrate composition of Fc and thus the conformation of the receptor binding epitope.

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REFERENCES

- Daeron, M. (1997) *Annu. Rev. Immunol.* **15**, 203–234
- Tamm, A., and Schmidt, R. E. (1997) *Int. Rev. Immunol.* **16**, 57–85
- Hulet, M. D., and Hogarth, P. M. (1994) *Adv. Immunol.* **57**, 1–127
- Galon, J., Robertson, M. W., Galinha, A., Mazieres, N., Spagnoli, R., Fridman, W. H., and Sautes, C. (1997) *Eur. J. Immunol.* **27**, 1928–1932
- Powell, M. S., Barton, P. A., Emmanouilidis, D., Wines, B. D., Neumann, G. M., Peitersz, G. A., Maxwell, K. F., Garrett, T. P., and Hogarth, P. M. (1999) *Immunol. Lett.* **68**, 17–23
- Kimberly, R. P., Ahlstrom, J. W., Click, M. E., and Edberg, J. C. (1990) *J. Exp. Med.* **171**, 1239–1255
- Unkeless, J. C., Shen, Z., Lin, C. W., and DeBeus, E. (1995) *Semin. Immunol.* **7**, 37–44
- Galon, J., Gauchat, J. F., Mazieres, N., Spagnoli, R., Storkus, W., Lotze, M.,

- Bonnefoy, J. Y., Fridman, W. H., and Sautes, C. (1996) *J. Immunol.* **157**, 1184–1192
9. Hulett, M. D., Witort, E., Brinkworth, R. I., McKenzie, I. F., and Hogarth, P. M. (1994) *J. Biol. Chem.* **269**, 15287–15293
 10. Hulett, M. D., Witort, E., Brinkworth, R. I., McKenzie, I. F., and Hogarth, P. M. (1995) *J. Biol. Chem.* **270**, 21188–21194
 11. Tamm, A., Kister, A., Nolte, K. U., Gessner, J. E., and Schmidt, R. E. (1996) *J. Biol. Chem.* **271**, 3659–3666
 12. Cook, J. P., Henry, A. J., McDonnell, J. M., Owens, R. J., Sutton, B. J., and Gould, H. J. (1997) *Biochemistry* **36**, 15579–15588
 13. Chappel, M. S., Isenman, D. E., Everett, M., Xu, Y. Y., Dorrington, K. J., and Klein, M. H. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 9036–9040
 14. Canfield, S. M., and Morrison, S. L. (1991) *J. Exp. Med.* **173**, 1483–1491
 15. Lund, J., Winter, G., Jones, P. T., Pound, J. D., Tanaka, T., Walker, M. R., Artymkiuk, P. J., Arata, Y., Burton, D. R., and Jefferis, R. (1991) *J. Immunol.* **147**, 2657–2662
 16. Henry, A. J., Cook, J. P., McDonnell, J. M., Mackay, G. A., Shi, J., Sutton, B. J., and Gould, H. J. (1997) *Biochemistry* **36**, 15568–15578
 17. Kato, K., Sautes-Fridman, C., Yamada, W., Kobayashi, K., Uchiyama, S., Kim, H., Enokizono, J., Galinha, A., Kobayashi, Y., Fridman, W. H., Arata, Y., and Shimada, I. (2000) *J. Mol. Biol.* **295**, 213–224
 18. Burmeister, W. P., Gastinel, L. N., Simister, N. E., Blum, M. L., and Bjorkman, P. J. (1994) *Nature* **372**, 336–343
 19. Garman, S. C., Kinet, J. P., and Jardetzky, T. S. (1998) *Cell* **95**, 951–961
 20. Sondermann, P., Huber, R., and Jacob, U. (1999) *EMBO J.* **18**, 1095–1103
 21. Maxwell, K. F., Powell, M. S., Hulett, M. D., Barton, P. A., McKenzie, I. F., Garrett, T. P., and Hogarth, P. M. (1999) *Nat. Struct. Biol.* **6**, 437–442
 22. Zhang, Y., Boesen, C. C., Radaev, S., Brooks, A. G., Fridman, W.-H., Sautes-Fridman, C., and Sun, P. D. (2000) *Immunity* **13**, 387–375
 23. Kato, K., Matsunaga, C., Igarashi, T., Kim, H., Odaka, A., Shimada, I., and Arata, Y. (1991) *Biochemistry* **30**, 270–278
 24. Kato, K., Matsunaga, C., Odaka, A., Yamato, S., Takaha, W., Shimada, I., and Arata, Y. (1991) *Biochemistry* **30**, 6604–6610
 25. Otwinowski, Z., and Minor, W. (1997) *Methods Enzymol.* **276**, 307–326
 26. Navaza, J. (1994) *Acta Crystallogr. Sect. A* **50**, 157–163
 27. Kissinger, C. R., Gehlhaar, D. K., and Fogel, D. B. (1999) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **55**, 484–491
 28. Brunger, A. T., Adams, P. D., Clore, G. M., Delano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, N., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **54**, 905–921
 29. Kleywegt, G. J., and Jones, T. A. (1996) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **52**, 829–832
 30. Deisenhofer, J. (1981) *Biochemistry* **20**, 2361–2370
 31. Harris, L. J., Larson, S. B., Hasel, K. W., Day, J., Greenwood, A., and McPherson, A. (1992) *Nature* **360**, 369–372
 32. Lawrence, M. C., and Colman, P. M. (1993) *J. Mol. Biol.* **234**, 946–950
 33. Sauer-Eriksson, A. E., Kleywegt, G. J., Uhlen, M., and Jones, T. A. (1995) *Structure* **3**, 265–275
 34. Corper, A. L., Sohi, M. K., Bonagura, V. R., Steinitz, M., Jefferis, R., Feinstein, A., Beale, D., Taussig, M. J., and Sutton, B. J. (1997) *Nat. Struct. Biol.* **4**, 374–381
 35. Harris, L. J., Larson, S. B., Hasel, K. W., and McPherson, A. (1997) *Biochemistry* **36**, 1581–1597
 36. Hibbs, M. L., Tolvanen, M., and Carpen, O. (1994) *J. Immunol.* **152**, 4466–4474
 37. Sondermann, P., Huber, R., Oosthuizen, V., and Jacob, U. (2000) *Nature* **406**, 267–273
 38. Garman, S. C., Wurzburg, B. A., Tarchevskaya, S. S., Kinet, J. P., and Jardetzky, T. S. (2000) *Nature* **406**, 259–266
 39. Jefferis, R., Lund, J., and Goodall, M. (1995) *Immunol. Lett.* **44**, 111–117
 40. Jefferis, R., Lund, J., and Pound, J. D. (1998) *Immunol. Rev.* **163**, 59–76
 41. Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998) *Nature* **395**, 82–86
 42. Davis, D. M., Chiu, I., Fassett, M., Cohen, G. B., Mandelboim, O., and Strominger, J. L. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 15062–15067
 43. Boyington, J. C., Motyka, S. A., Schuck, P., Brooks, A. G., and Sun, P. D. (2000) *Nature* **405**, 537–543
 44. Burmeister, W. P., Huber, A. H., and Bjorkman, P. J. (1994) *Nature* **372**, 379–383
 45. Ravetch, J. V., and Clynes, R. A. (1998) *Annu. Rev. Immunol.* **16**, 421–432
 46. Boros, P., Odin, J. A., Chen, J., and Unkeless, J. C. (1994) *J. Immunol.* **152**, 302–306